To: Office of the Commissioner of Agriculture
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Re: Final Report: Functional Foods and Nutraceutical Development from Indiana Agricultural Products

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Goals

There were three goals for the research project:

1. Develop method for lycopene extraction from waste tomato skins using supercritical fluid extraction
2. Design three different functional food ingredients containing lycopene, soy oil, lecithin and genistein
3. Quantitate lycopene and vitamin E levels

Overall Conclusion

All three goals were successfully completed. Specific details of the lycopene extraction and lycopene, vitamin E quantitation can be found in Journal Agricultural and Food Chemistry, 2002, Rozzi, Singh, Vierling and Watkins. Most importantly the nutraceuticals were successfully encapsulated into useable forms, both wet and dry. These studies showed that new functional food ingredients could be made from Indiana agricultural products.

Experiment results for goals 1 and 3 (lycopene extraction, and lycopene and vitamin E quantitation).

Material and Methods

Sample Preparation

Tomato Seeds and Skins: Tomato seeds and skins (51.6 % dry matter) were obtained from Red Gold, Inc (Elwood, IN) on September 13, 2000. The samples were the by-product of steam pealing used to produce tomato sauce and collected prior to removal from the processing facility. The sample was stored at –20 °C until used and did not undergo any further preparation. The distribution of seeds and skins within 3 g of the raw material was 30.5±2.2 % tomato skin and 69.5±2.2 % tomato seeds.

Chemical Extraction

A chemical extraction of lycopene from tomato by-product was performed to serve as a standard for the recovery of phytochemicals in the test material. A 2 g sample of seeds and skins was placed in an extraction tube and 20 ml of chloroform added to the tube followed by sonication for 30 min. The sample was centrifuged for 15 min at 2000 rpm (913 x g) and an aliquot removed for analysis of lycopene content by HPLC. The extraction procedure was repeated on the sample to recover residual lycopene in the sample. Exhaustive extraction of tomato seeds and skins with additional volumes of chloroform did not result in additional recovery of lycopene.

Supercritical Fluid Extraction

The supercritical fluid extraction (SFE) system consisted of an Isco Model 260 D syringe pump, SFX-210 extractor, and a temperature controlled variable restrictor (Isco, Lincoln, NE). Three experiments were conducted to assess the effects of temperature, pressure, flow rate, and CO_2 volume on SFE of phytochemicals from the samples. The experiments evaluated the following: experiment 1 (Exp. 1) the effect of CO_2 temperature and pressure, experiment 2 (Exp. 2) the effect of CO_2 flow rate, and experiment 3 (Exp. 3) the effect of CO_2 volume. Exp. 1 provided information on factors that contributed to improved solubility, Exp. 2 evaluated flow rate for CO_2, and Exp. 3 tested the combination of operation factors on recovery of lycopene from tomato seeds and skins.

The temperatures tested in Exp. 1 ranged from 32 to 86 °C at 9 °C intervals, and the pressures from 13.78 to 48.26 MPa at 3.45 MPa intervals. The flow rate of the CO_2 was maintained at 2.5-ml/ min for 20 min, giving a total volume of 50 ml of CO_2. After the extraction, the sample was removed, and another 50 ml of CO_2 was passed through the extractor to ensure that any remaining lycopene was recovered. The flow rates were measured at the extraction temperature and pressure due to equipment design. The conditions were fully crossed in the factorial design, and the extractions were performed in triplicate. To prevent plugging, the restrictor temperature was maintained at 15 °C above the extraction temperature. Extracts were collected in a vial immersed in ice water to facilitate condensation of the extract. Once collected, extracts were dissolved in 5 ml of MTBE (Methyl *tert*-Butyl Ether) for analysis by HPLC.

The extractions for Exp. 2 were performed by holding the temperature, pressure, and CO_2 volume constant and then increasing the flow rate of CO_2 used for each extraction. The temperature and pressure selected for Exp. 2 was the optimum conditions determined for the extraction of lycopene in Exp. 1. For this experiment, flow rates from 2.5 ml/min to 15 ml/min were examined. These flow rates were tested at intervals of 2.5 ml/min. The optimum conditions determined in Exp. 1 and 2, were utilized in Exp. 3 to determine the effect of CO_2 volume on the extraction of lycopene under optimum temperature and pressure conditions. This was accomplished by repeatedly collecting extracts from a sample at 100 ml intervals until a total of 1200 ml of CO_2 was used to perform the extraction.

Carotenoid and Tocopherol Determination

The concentrations of lycopene, β-carotene, α-carotene, α-tocopherol, γ-tocopherol, and δ-tocopherol in each extract were quantified using an HPLC equipped with an electro-chemical detector (Coularray, ESA, Inc., Chelmsford, MA). Two mobile

phases were used for the gradient separation [mobile phase A (pH 4.8): methanol: ammonium acetate (0.2 M) (90:10) and mobile phase B (pH 4.8): methanol: 1-propanol: ammonium acetate (1.0 M) (78:20:2)]. The gradient used for this separation consisted of 100% mobile phase A at time 0, 80% mobile phase B at 10 min, 100% mobile phase B at 20 min, 100% mobile phase B at 27 min, 100% mobile phase A from 28 min through 32 min. All transitions between mobile phases were linear. The flow rate for the separation was 1.6 ml/min, which generated a pressure of 14 MPa. A Phenomenex Luna C-18 (2) column was used for the separation (150 mm x 4.6 mm, 3 µm particle size) (Phenomenex, Inc., Torrance, CA). The cell potentials for the detector were set at 350-700 mV by increments of 50 mV and the column was maintained at a temperature of 37 °C. Carotenoid standards were purchased from Sigma (St. Louis, MO) and tocopherol standards were purchased from Matraya (Pleasant Gap, PA).

Results

Figures 1 and 2 show how changes in extraction parameters affect the yield of lycopene from tomato by-products.

Figure 1. Concentration of lycopene extracted from tomato seeds and skins vs. extraction pressure at different temperatures.

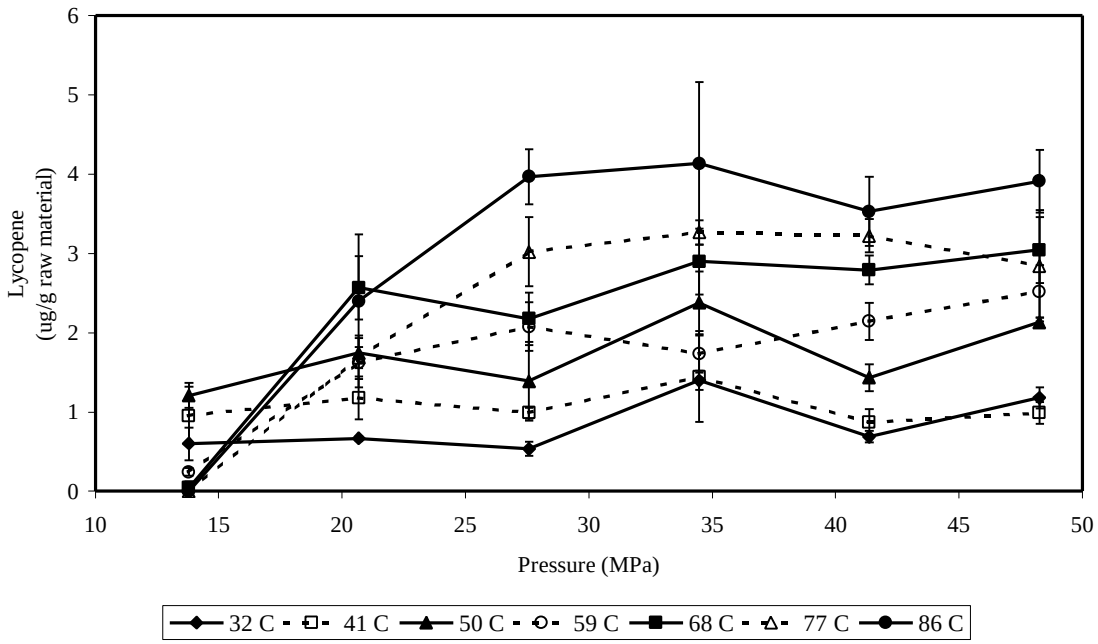


Figure 2. Concentration of lycopene extracted from tomato seeds and skins at 86°C and 34.47 MPa vs. flow rate of CO_2.

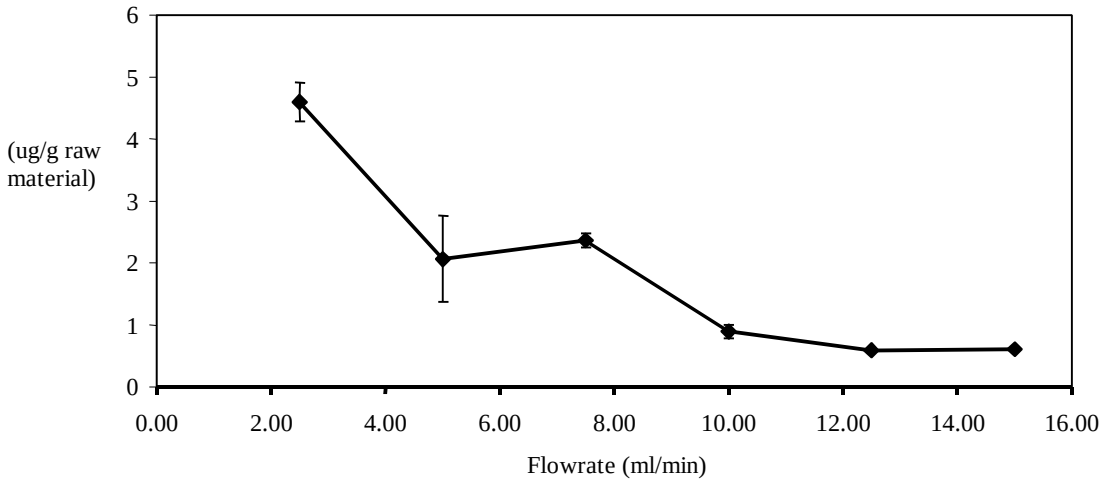
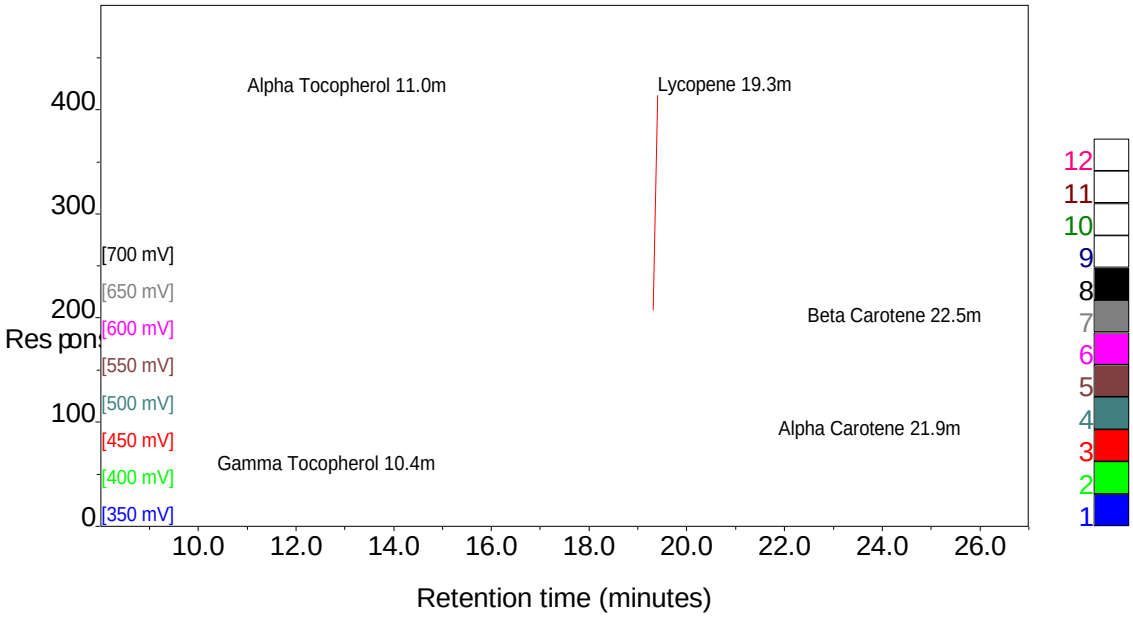


Figure 3 shows that we were able to successfully quantitate the amounts of lycopene and vitamin E in the samples.

Fig. 3 Chromatogram from an HPLC outfitted with electrochemical detection of a tomato extract produced by supercritical CO_2 extraction at 50 °C and 13.7 MPa



Experiment results for goal 2 (encapsulation of lycopene so that it could be used as a functional food ingredient or mixed with encapsulated genistein and made into a new ingredient).

Figures 4 thru 9 are photographs of various formulations of encapsulated lycopene in both dry and wet states.